EMERGENCY USE AUTHORIZATION (EUA) SUMMARY
The Gravity Diagnostics SARS-CoV-2 RT-PCR for use with DTC kits
Gravity Diagnostics, LLC

For *In vitro* Diagnostic Use
For use under Emergency Use Authorization (EUA) only

The Gravity Diagnostics SARS-CoV-2 RT-PCR for use with DTC kits will be performed at Gravity Diagnostics, LLC, certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. §263a, and meets the requirements to perform high complexity tests, as described in the standard operating procedure that was reviewed by the FDA under this EUA.

INTENDED USE

1) Intended Use

The Gravity Diagnostics SARS-CoV-2 RT-PCR for use with DTC kits is a direct to consumer product for testing of anterior nasal swab specimens self-collected at home using either: (1) the Everlywell COVID-19 Test Home Collection Kit DTC by any individuals, 18 years or older, including individuals without symptoms or other reasons to suspect COVID-19; or (2) the Kroger Health COVID-19 Test Home Collection Kit either unobserved or video-observed by any individuals, 16 years or older, including individuals without symptoms or other reasons to suspect COVID-19.

Testing of self-collected anterior nasal swab specimens is limited to Gravity Diagnostics, LLC, located at 632 Russell Street, Covington, KY 41011 and 812 Russell Street, Covington, KY 41011, which are certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. §263a, and meet requirements to perform high complexity tests.

Results are for the identification of SARS-CoV-2 RNA. The SARS-CoV-2 RNA is generally detectable in anterior nasal swab specimens during the acute phase of infection. Positive results are indicative of the presence of SARS-CoV-2 RNA; clinical correlation with patient history and other diagnostic information is necessary to determine patient infection status. Positive results do not rule out bacterial infection or co-infection with other viruses. The agent detected may not be the definite cause of disease. Laboratories within the United States and its territories are required to report all results to the appropriate public health authorities. Negative results do not preclude SARS-CoV-2 infection.

Use of your product is not a substitute for visits to a healthcare provider. The information provided by this product should not be used to start, stop, or change any course of treatment unless advised by your healthcare provider.

Testing with the Gravity Diagnostics SARS-CoV-2 RT-PCR is intended for use by qualified clinical laboratory personnel specifically instructed and trained in the techniques of real-time

PCR and in vitro diagnostic procedures The assay is only for use under the Food and Drug Administration's Emergency Use Authorization.

2) Special Conditions of Use Statements

For *in vitro* diagnostic use only
For Emergency Use only

This assay can be used with the Everlywell COVID-19 Test Home Collection Kit DTC. Everlywell has granted Gravity Diagnostics, LLC a right of reference to the data supporting use of this collection kit.

This assay can be used with the Kroger Health COVID-19 Test Home Collection Kit. The Kroger Co. has granted Gravity Diagnostics, LLC a right of reference to the data supporting use of this collection kit.

DEVICE DESCRIPTION AND TEST PRINCIPLE

The Gravity Diagnostics SARS-CoV-2 RT-PCR for use with DTC kits is a real-time reverse transcription polymerase chain reaction (rRT -PCR) test. The test uses three primer and probe sets to detect one region in the N gene, one region in the ORF1ab, and one region in the S gene, as well as one additional primer and probe set to detect an MS2 internal control, added to each clinical sample before extraction. It also contains one primer and probe set to detect human RNase P (RP) in a clinical sample. The primers/probes, used to detect all targets, are combined in the same reaction well. RNA is isolated using the Thermo Fisher MagMAX Viral/Pathogen II (MVP II) Nucleic Acid Isolation Kit from anterior nasal swabs and is reverse transcribed to cDNA and subsequently amplified using an Applied Biosystems QuantStudio7 Flex instrument (QS7) with software version 1.3 or a QuantStudio12 Flex (QS12) instrument with software version 1.2.2. During the amplification process, each probe anneals to a specific target sequence located between the forward and reverse primers. During the extension phase of the PCR cycle, the 5' nuclease activity of Taq polymerase degrades the bound probe, causing the reporter dye[1] to separate from the quencher dye, generating a fluorescent signal. Fluorescence intensity is monitored at each PCR cycle by the QS7 or QS12 instruments.

INSTRUMENTS USED WITH THE TEST

The Gravity Diagnostics SARS-CoV-2 RT-PCR for use with DTC kits is to be used with the Thermo Fisher KingFisher Flex with software version 2.5 and the Hamilton Microlab STAR with software version 1.01 automated nucleic acid isolation instruments. The RT-PCR occurs on the Thermo Fisher QuantStudio 12K Flex and QuantStudio 7. The software version on the QuantStudio 12K Flex is V1.2.2 from Applied Biosystems and on the QuantStudio 7 is version 1.3 from Applied Biosystems.

[1] Target-reporter dye pairs are as follows: (1) ORF1ab-FAM, (2) N gene-VIC, (3) S gene-ABY, (4) MS2-JUN, (5) RP-Cy5

REAGENTS AND MATERIALS

1) Included with the Assay

Equipment/Reagents/Consumables	Catalog #	Manufacturer
TaqPath™ 1 Step Multiplex Master Mix (No ROX™) (4X)	A28522	ThermoFisher
TaqPath™ COVID-19 Combo Kit	A47814	Applied Biosystems™
Thermo Fisher MagMAX Viral/Pathogen II (MVP II) Nucleic Acid Isolation Kit	A48383	ThermoFisher
RNase P Primer F	custom	IDT/Eurofins
RNase P Primer R	custom	IDT/Eurofins
RNase P Primer Probe	custom	IDT/Eurofins

2) Not Included with the Assay

Equipment/Reagents/Consumables	Catalog #	Manufacturer
King Fisher Flex Purification System	24074421	ThermoFisher
Hamilton Microlab STAR	A42352	Hamilton Robotics
Fisher accuSpin Micro17	75002461	ThermoFisher
Sorvall T1 centrifuge	750002382	ThermoFisher
MiniVortex	NA	NA
Quant Studio 12 Flex	4471086	Applied Biosystems
Quant Studio 7 Flex	4485701	Applied Biosystems
Magmax 96 standard well plates 200ul	97002540	Life Technologies
Magmax tip comb	97002534	Life Technologies
Magmax 96 deep well plates	95040460	Life Technologies
50mL conical tube	12565271	Fisher
15mL conical tube	1495953A	Fisher
384 well PCR plates	4309849	Fisher
Adhesive PCR film	4311971	Fisher
Tips ranging from 1.0ul to 1000ul	NA	Integra and USA Scientific
10mL serological pipettes	1071-0810	Fisher
0.2 strip tubes, 8 well	AB2000	Fisher

CONTROLS TO BE USED WITH THE TEST

1. **Positive Control (PC)**: The positive control is run on every plate. The positive control is a diluted mix of Thermo Fisher TaqPath COVID-19 Control (25 copies/uL) with Hs-RPP30 Positive Control (Integrated DNA Technologies, CAT#: 10006626; 2,000 copies/uL). The TaqPath COVID-19 control contains the sequence for the three SARS-CoV-2 assays, while the Hs-RPP30 control contains the sequence for the RNase P assay. This control monitors amplification and signal production and ensures the integrity of the PCR reagents.

2. **Negative (No Template) Control (NTC)**: The negative control is run on every plate. The negative control is blank extraction reagents without target nucleic acid that are extracted and processed in the real time RT-PCR along with the patient samples. This control monitors for contamination during the extraction process and in the real time RT-PCR reagents.
3. **Internal Control 1 (MS2)**: The MS2 internal control RNA is added to each sample prior to nucleic acid isolation and is run for every sample. The MS2 is a bacteriophage RNA target included in the TaqPath COVID-19 Combo Kit (Applied Biosystems, CAT #: A47814). This control monitors for nucleic acid extraction, reverse transcription, amplification and signal production in each sample.
4. **Internal Control 2 (RNase P):** This internal control is human RNase P gene and is run with every sample. This control monitors for nucleic extraction and ensures sample integrity of the human specimen collected for testing.

INTERPRETATON OF RESULTS

All test controls should be examined prior to interpretation of patient results. If the controls are not valid, the patient results cannot be interpreted.

Table 1. Expected Results for the Gravity Diagnostics SARS-CoV-2 RT-PCR for use with DTC kits Controls

Control	Expected Results (Expected Ct)				
	N target	Orf1ab target	S Target	MS2	RNAse P
Positive Control	Positive (Ct < 38)	Positive (Ct < 38)	Positive (Ct < 38)	Negative (Ct ≥ 38)	Positive (Ct < 38)
No Template Control	Negative (Ct ≥ 38)	Negative (Ct ≥ 38)	Negative (Ct ≥ 38)	Negative (Ct ≥ 38)	Negative (Ct ≥ 38)

Assessment of clinical specimens must be performed after the positive, negative (no template), and internal controls (RNase P and MS2) have been examined and determined to be valid. If the positive or negative controls are not valid, the patient results cannot be interpreted.

Note on the internal controls: Samples that test positive for SARS-CoV-2 targets do not require amplification of the internal controls to be valid. However, amplification of the RNase P internal control is required for a negative SARS-CoV-2 sample to be valid.

The interpretation and reporting of clinical specimens are summarized in Table 2.

Table 2. Result Interpretation for Patient Samples

Orf1ab	N Gene	S Gene	MS2	RNAse P	Result	Action*
NEG (Ct ≥ 38)	NEG (Ct ≥ 38)	NEG (Ct ≥ 38)	NEG (Ct ≥ 38)	NEG (Ct ≥ 38)	Invalid	Repeat test. If the repeat result remains invalid, if the repeated result remains invalid, request a new specimen from

Orf1ab	N Gene	S Gene	MS2	RNAse P	Result	Action*
						the individual.
NEG (Ct ≥ 38)	NEG (Ct ≥ 38)	NEG (Ct ≥ 38)	POS (Ct < 38)	NEG (Ct ≥ 38)	Invalid	Request a new specimen from the individual.
NEG (Ct ≥ 38)	NEG (Ct ≥ 38)	NEG (Ct ≥ 38)	POS or NEG	POS (Ct < 38)	SARS-CoV-2 Not Detected	Report "not detected" to appropriate public health authorities and individual*.
Only one SARS-CoV-2 target POS (Ct < 38)			POS or NEG	POS or NEG	SARS-CoV-2 Inconclusive	Repeat test. If the repeat result remains inconclusive, report "inconclusive" to appropriate public health authorities and individual* and request an additional specimen be collected for testing.
Two or more SARS-CoV-2 targets = POS (Ct < 38)			POS or NEG	POS or NEG	SARS-CoV-2 Positive	Report "detected" to appropriate public health authorities and individual*.

*For at home collection from Everlywell, reporting will be done via Gravity Diagnostics' Laboratory Information System (LIS) to Physicians Wellness Network (PWN) . For details on this process, please refer to Everlywell COVID-19 Test Home Collection Kit DTC EUA summary. For at home collection from Kroger, reporting will be done via Gravity Diagnostics' LIS to Kroger Health. For details on this process please refer to the Kroger Health COVID-19 Test Home Collection Kit EUA summary. These summaries can be found at https://www.fda.gov/medical-devices/coronavirus-disease-2019-covid-19-emergency-use-authorizations-medical-devices/vitro-diagnostics-euas.

SAMPLE INTAKE - INSPECTION OF SAMPLES

Specimen Accessioning for Authorized DTC Collection Kits:

For the Everlywell COVID-19 Test Home Collection Kit DTC specimen accessioning will be performed via the Everlywell SOP "Receiving and Processing Everlywell Samples". For the Kroger Health COVID-19 Test Home Collection Kit specimen accessioning will be performed via "The Kroger Co. COVID-19 Lab Accessioning SOP".

PERFORMANCE EVALUATION

(The performance of the Gravity Diagnostics SARS-CoV-2 RT-PCR for use with DTC kits (EUA21034) is the same data used to the support the previous authorization of the Gravity Diagnostics SARS-CoV-2 RT-PCR Assay (EUA202301) - which represent the same real-time RT-PCR test used for different indications of use.)

1) Analytical Sensitivity

1a) Limit of Detection (LoD)

The details of the LoD study are available in the FDA EUA "the Gravity Diagnostics SARS-CoV-2 RT-PCR Assay".

The LoD for the Gravity Diagnostics SARS-CoV-2 RT-PCR Assay for use with DTC kits is 500 GE/mL using the Hamilton extraction platform and 1000 GE/mL using the KingFisher extraction platform.

1b) Inclusivity

The primer/probe set for the ORF1ab, N, and S SARS-CoV-2 targets were designed by ThermoFisher, which conducted the *in silico* inclusivity analysis. The data from this analysis is available in the FDA EUA "TaqPath COVID-19 Combo Kit". A right of reference letter was obtained by Gravity Diagnostics for these data.

FDA performed an *in silico* analysis of the Gravity Diagnostics SARS-CoV-2 RT-PCR for use with DTC kits primer/probe sets, which identified the B.1.1.7 variant (England), as being most likely to impact the S target detection. The initial risk assessment determined that because the other two targets are expected to not be affected, no further action is needed.

2) Analytical Specificity - Cross-reactivity

The primer/probe set for the ORF1ab, N, and S SARS-CoV-2 targets were designed by ThermoFisher, which conducted the cross-reactivity studies. The data from this analysis is available in the FDA EUA "TaqPath COVID-19 Combo Kit". A right of reference letter was obtained by Gravity Diagnostics for these data.

3) Clinical Evaluation

The details of the clinical evaluation are available in the FDA EUA "the Gravity Diagnostics SARS-CoV-2 RT-PCR Assay".

The performance of the Gravity Diagnostics SARS-CoV-2 RT-PCR for use with DTC kits is shown in Table 3.

Table 3. Clinical Performance of the Gravity Diagnostics SARS-CoV-2 RT-PCR for use with DTC kits.

		EUA-authorized RT-PCR Assay Comparator		
		Positive	**Negative**	**Total**
Gravity Diagnostics SARS-CoV-2 RT-PCR	**Positive**	53	0	53
	Negative	2	115	117
	Total	55	115	170
Positive Agreement		96.4% (53/55) (95% CI 87.7 –99.0)		
Negative Agreement		100.0% (115/115) (95% CI 96.8 – 100.0)		

4) Performance among individuals without symptoms or other reasons to suspect COVID-

The details of the clinical evaluation among individuals without symptoms or other reasons to suspect COVID-19 are available in the FDA EUA "the Gravity Diagnostics SARS-CoV-2 RT-PCR Assay".

The performance of the Gravity Diagnostics SARS-CoV-2 RT-PCR for use with DTC kits among individuals without symptoms or other reasons to suspect COVID-19 is shown in Table 4.

Table 4. Clinical Performance of the Gravity Diagnostics SARS-CoV-2 RT-PCR for use with DTC kits among Asymptomatic Individuals.

		EUA-authorized RT-PCR Assay Comparator		
		Positive	**Negative**	**Total**
Gravity Diagnostics SARS-CoV-2 RT-PCR	**Positive**	20	0	20
	Negative	0	100	100
	Total	20	100	120
Positive Agreement		100.0% (20/20) (100% CI 83.9 – 100.0)		
Negative Agreement		100.0% (100/100) (95% CI 96.3 – 100.0)		

FDA SARS-CoV-2 Reference Panel Testing

The details of the FDA SARS-CoV-2 Reference Panel Testing are available in the FDA EUA "the Gravity Diagnostics SARS-CoV-2 RT-PCR Assay". The Product LoD of the Gravity Diagnostics SARS-CoV-2 RT-PCR for use with DTC kits is $1.8x10^4$ NDU/mL.

LIMITATIONS:

- Detection of RNase P indicates that human nucleic acid is present and implies that human biological material was collected and successfully extracted and amplified. It does not necessarily indicate that the specimen is of appropriate quality to enable detection of SARS-CoV-2.
- The clinical performance has not been established in all circulating variants but is anticipated to be reflective of the prevalent variants in circulation at the time and location of the clinical evaluation. Performance at the time of testing may vary depending on the variants circulating, including newly emerging strains of SARS-CoV-2 and their prevalence, which change over time.

WARNINGS:

- For Emergency Use Authorization (EUA) only.
- For in vitro diagnostic use.
- This product has not been FDA cleared or approved but has been authorized for emergency use by FDA under an EUA for use by authorized laboratories.
- This product has been authorized only for the detection of nucleic acid from SARS-CoV-2, not for any other viruses or pathogens.
- The emergency use of this product is only authorized for the duration of the declaration that circumstances exist justifying the authorization of emergency use of in vitro diagnostics for detection and/or diagnosis of COVID-19 under Section 564(b)(1) of the Federal Food, Drug and Cosmetics Act, 21 U.S.C. § 360bbb-3(b)(1), unless the declaration is terminated or authorization is revoked sooner.